

## Characterization of Haragauri Rasa and Its Anti-Microbial Activity on Pneumonia Causing Organisms

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**ABSTRACT:Background:** This article shares a step in pharmaceutical progress for an uncommon Herbo-mineral Ayurvedic Koopipakva Rasayana termed as Haragauri Rasa for its anti-microbial study on Pneumonia (Shwasanaka Jwara) causing organisms. **Materials & Methods:** Literature review on required divisions. Parada-Gandhaka-Naavasadara Shodhanam. Followed by the Tritiyamsha Kajjali Nirmanam and addition of Shuddha Navasadara. Later Mardana with Dhatura Patra Rasa and finally Antardhooma Koopipaka for 16 Yama (48 hours). **Results:** Organoleptic, physicochemical, instrumental and anti-microbial analysis were done. **Discussion:** Importance of Paratantra Avalokanam on multiple steps, comparison with previously done works and Law of theoretical yield with its importance and interpretation was elucidated. **Conclusion:** Haragauri Rasa having 200nm particle size along with its effectiveness as an anti-bacterial and anti-fungal compound against Pneumonia causing organisms shows the existence of nanomedicine in Ayurveda and gives a potential Aushadha for any Atyayika-Chikitsa emergency condition.

**Keywords:** Haragauri Rasa, Koopipakva, Antardhooma, Swasanaka Jwara, Pneumonia, Nanomedicine, Ayurveda nanomedicine, anti-microbial, anti-fungal

### I. INTRODUCTION:

The present article shares the gist of a complete dissertation work which was taken up as per the notice Ref. No 26-74/2021-(Common matter), dated 04.05.2021 from the Rajiv Gandhi University of Health Sciences, Bengaluru, Karnataka; addressed to the Principal/Dean/Directors of

Ayurveda/Unani/Siddha & Sowa Rjgpa colleges, the focus for dissertation work in ASU institutions should be exclusively on different aspects of COVID-19 situation. Hence, a classical Koopipakva Rasayana given as Haragauri Rasa, currently unavailable in market was taken up to check its anti-microbial activity on one of the major diseases Pneumonia, also happened to be one of the etiological factors in manifesting COVID-19[1] which can be co-related as Jivanuvishajanya Swasanaka Jwara.[2]

Haragauri Rasa is one such Koopipakva formulation from Rasa Kamadhenu[3], indicated in Vatavyadhi but this same formulation by Rasa Sanketa Kalika[4] is indicated for Dhatu Kshaya, Jirna Jwara, Prameha, Vrushya, Sarva Vyadhi with respective Anupana and author of Rasa Tantra Sara Va Siddha Prayoga Sangraha[5] quotes its indications same as Rasa Sindooram[6], which has Swasahara, Kasahara, Sarva Jwarahara, Krimighna properties too.

The main aim of the study was the pharmaceutical preparation of Haragauri Rasa as per the classical reference of Rasa Kamadhenu with Tritiyamsha Kajji in 16 Yama Paka Kala and evaluate its anti-microbial activity on *S. aureus*, *K. pneumoniae* and anti-fungal activity on *C. albicans* by in-vitro disc-diffusion method.

### II. MATERIAL & METHODS:

**Literature review:** It contains mainly disease review since the work is focused on particularly pneumonia causing organisms, its probable Ayurvedic correlation and Ayurvedic point of view of the relevant disease. It also explains drug review of all the Rasa Dravya and Kashtha Dravya; its origin, synonyms, Guna-Karma, indications.

Adding to that, it also has formulation review for an insight to understand the periphery of the science of Koopipakva Nirmana.

Pharmaceutical study: It contains the SOP and required technicalities of performed practicals, observations and precautions.

**Table 1: Performed practicals in order to complete the pharmaceutical study**

Name of the practical	Elected method	Ingredients	Duration	Initial weight	Final weight
Parada Shodhanam	Urdhvapatana Vidhi[7]	Ashuddha Parada – 1000g Haridra Churna – 250g Kumari Rasa – QS (1114.3g)	12 hours Mardana 5 hours Patana	1000g	962g (Loss of 38g)
Gandhaka Shodhana	Dhalana Vidhi[8]	Ashuddha Gandhaka – 125g Shveta Bhrungaraja Rasa – 630g Go Ghruta – QS (50g)	7 times Dhalana	125g	114g (Loss of 11g)
Navasagara Shodhana	Nirjalikarana Vidhi[9]	Ashuddha Navasagara – 300g Jalam – 900g	Till complete evaporation of Jala	300g	281g (Loss of 19g)
Kajjali Nirmanam	Nirdrava Mardanam[10]	Shuddha Parada – 300g Shuddha Gandhaka – 100g	Tapta Khalva Mardana – 22 hours Normal Ashma Khalva – 19 hours	400g	389g (Loss of 11g)
Dattura Patra Rasa Mardana & Navasagara Prakshepa	Sadrava Mardanam[11]	Parada Gandhaka Tritiyamsha Kajjali – 126g Dattura Patra Swarasa – QS (20g) Shuddha Navasagara – 10g	Mardana for 1 Yama (3 hours) and sundried Navasagara Prakshepa in dry Kajjali and immediately shifted in Koopi and complete corking done	126g	136g (Gain of 10g)
Koopipaka of Haragauri Rasa	Antardhooma Koopipakva Nirmanam[12]	Dhattura Patra Swarasa Mardita Navasara Prakshepita Parada Gandhaka	16 Yama (48 hours) Mrudu Agni	136g	73.5g (Loss of 62.5g)

		Trutiyamsha Kajjali – 136g	– 5.5 Yama (16 hours) [Room temp. - 292°C at bottom of Loha Nandi]  Madhyama Agni – 8 Yama (24 hours) [500°C at bottom of Loha Nandi]  Tivra Agni – 2.5 Yama (8 hours) [846°C at bottom of Loha Nandi]		[Madhyastha Product]
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**Table 2: Temperature Pattern details**

Time	Temp at 2 Angula Below Koopi Kantha	Koopi	Bottom of Nandi
<b>DAY 1</b>			
07:15 AM	28°C	29°C	77°C
08:00 AM	28°C	33°C	120°C
09:00 AM	28°C	43°C	125°C
10:05 AM	40°C	57°C	154°C
11:00 AM	40°C	61°C	166°C
12:00 PM	50°C	66°C	175°C
01:00 PM	57°C	67°C	180°C
02:00 PM	60°C	76°C	200°C
03:00 PM	66°C	80°C	212°C
04:00 PM	72°C	88°C	229°C
05:00 PM	75°C	86°C	240°C
06:00 PM	81°C	92°C	252°C



07:00 PM	86°C	97°C	280°C
08:00 PM	90°C	100°C	287°C
09:00 PM	87°C	94°C	290°C
10:00 PM	90°C	95°C	285°C
11:00 PM	92°C	96°C	292°C
12:00 AM	96°C	95°C	294°C
01:00 AM	80°C	94°C	309°C
02:00 AM	88°C	95°C	320°C
03:00 AM	89°C	100°C	260°C
04:00 AM	88°C	102°C	275°C
05:00 AM	95°C	104°C	288°C
06:00 AM	96°C	108°C	302°C
<b>DAY 2</b>			
07:00 AM	95°C	105°C	337°C
08:00 AM	101°C	110°C	357°C
09:00 AM	107°C	120°C	362°C
10:00 AM	100°C	111°C	352°C
11:00 AM	108°C	112°C	350°C
12:00 PM	104°C	115°C	319°C
01:00 PM	111°C	120°C	363°C
02:00 PM	115°C	125°C	353°C
03:00 PM	114°C	122°C	371°C
04:00 PM	117°C	123°C	377°C
05:00 PM	119°C	123°C	384°C
06:00 PM	125°C	124°C	380°C

07:00 PM	124°C	128°C	394°C
08:00 PM	127°C	142°C	400°C
09:00 PM	128°C	140°C	480°C
10:00 PM	136°C	142°C	505°C
11:00 PM	128°C	147°C	483°C
12:00 AM	143°C	156°C	519°C
01:00 AM	137°C	147°C	523°C
02:00 AM	110°C	146°C	613°C
03:00 AM	141°C	161°C	619°C
04:00 AM	134°C	157°C	668°C
05:00 AM	140°C	157°C	707°C
06:00 AM	142°C	161°C	846°C

(Note: Extreme bottom of the Nandi had turned into full red-hot colour and showed 900°C)

### III. RESULTS:

Analytical study: It contains organoleptic, physicochemical, instrumental and anti-microbial results given in following tables.

**Table 3: Analytical results**

	Parameters	Results
<b>Organoleptic results</b>	Colour	Asita Rakta Sindoor Varna
	Odour	Characteristic
	Taste	Tasteless
	Appearance/Touch	Sukshma Snigdha Churna
<b>Physicochemical results</b>	Moisture content	0.986
	Loss on drying	0.163
	Ash Value	14.62
	Acid soluble ash	3.096
	Water soluble ash	2.621
	pH value	8.27
<b>Instrumental results</b>	XRD	HgS with crystalline nature, hexagonal lattice with 200nm particle size
	SEM	Morphology of the particles are irregular shape and highly porous
	EDAX	Presence of Sulphur and Mercury only

**Table 4: Anti-microbial results**

Inhibition zone (mm)				
Sl No.	Concentration(µg/mL)	K. pneumoniae	S. aureus	C. albicans
a	5	6±0.20	4±0.20	No zone of inhibition
b	10	8±0.40	9±0.20	2±0.20
c	25	10±0.40	13±0.20	4±0.40
d	50	19±0.20	22±0.40	7±0.40
e	Std.	17±0.20	19±0.30	14±0.40
<b>Standard: Bacteria:</b> Amoxicillin (10 µg/mL); <b>Fungus:</b> Gentamycin (10 µg/mL). Experiments were done in triplicates and mean values were taken.				
	<b>MIC concentration</b>	3.8 µg/mL	4.6 µg/mL	7.6 µg/mL

#### IV. DISCUSSION:

**A.) Importance of Paratantravalokanam:** As Acharya Sushruta has advised the Vaidya to have both the qualities in them which is to be Swatantrakushala[13] & do Paratantravalokanam.[14] This concept of Paratantravalokanam was very helpful in the present work as the main references taken here for various procedures were having Nigudha and Lesha Ukta. Which can be listed as below:

##### Parada Shodhanam:

- ❖ Parada, Haridra Churna and Kumari Rasa. The ratio was missing → Parada Samhita[15] 1/4 or 1/8 Parts of the Shodhana Dravya.
- ❖ The duration of Mardana has been told Eka Dina → 12 hours as well as 24 hours → no mentioning of Ratri and hence 12 hours was decided.
- ❖ Only mentioning of Patana Vidhi but which type? → Rasendra Sarasangraha,[7] Bharata Bhaishajya Ratnakara[16] and Samskruta Teeka of Ayurveda Prakasha[17] helped to finalise for the Urdhvapatana Vidhi.
- ❖ Another doubt was → Duration and type of Agni of Urdhvapatana Vidhi → Idea for duration was taken from previous dissertation works, fixed as 5 hours and Kramagni[18] was determined from Parada Samhita.

##### Kajjali Nirmanam:

- ❖ Commonly the Kajjali Nirmanam has been done with the help of Ashma Khalva. However, the Shastra emphasis the Tapta Khalva for Parada Pradravana[19] and also because of availability of Vidhi for Gandhaka Jarana in Tapta Khalva[20] an attempt was made to work around these concepts and hence

Tapta Khalva was taken for Kajjali Nirmanam and achieved it as well.

##### Koopipaka Jarana Vidhi:

- ❖ Rasakamdhenu author mentioned → Paka should be done according to Gurukta Sampradaya for 16 Yama (48 hours).
- ❖ Classical references were crosschecked to follow a Sampradaya → Rasayana Sara Antardhooma Chandrodaya Nirmana Vidhi[21] was picked.
- ❖ Type of Gandhaka Jarana → Rasayana Sara → suggesting all the Chandrodaya Rasa etc. mentioned in Bahirdhooma Paka can be prepared in Antardhooma Vidhi as well.[22]
- ❖ Along with that, from Rasamanjari Rasasindoor Nirmana Vidhi[23] navigation was secured to do Antardhooma Paka for long durations like 36 hours and more.
- ❖ Besides, if Bahirdhooma Paka is done then the chances of Gandhaka Dhooma Sevana for a very long duration are possible which can lead to Vikara and therefore, Antardhooma Paka was firmly decided.

##### Tapamana Krama:

- ❖ The main reference quotes Manda-Madhyahathagni for 16 Yama but how to bifurcate the total duration and in which ratio was a mystery and it got resolved after reading Parada Vijnaniyam saying Mrudu agni for 6-8 hours, Madhyama Agni for 8 hours and Tivra Agni for 4/8/10 hours.[24]
- ❖ Based on this, temperature classification was done as 16 hours Mrudvagni, 24 hours Madhyamagni and 8 hours Tikshnagni.
- ❖ Temperature versus time graph was done prior to the Paka on the understanding based on Parada Vijnaniyam.[24]

Duration of Agnipaka:

- ❖ As per the reference mentioned in Parada Samhita[25] 12 Yama (36 hours) Paka should be done when 1 Pala (48g) of Parada is taken and more Agni should be given for more amount with more intensity. Here in this practical, total amount of Kajjali taken was 136g containing 84.42g of Parada and therefore total 16 Yama Paka was finalised.

**B.) Comparison of Pharmaceutical procedures with Previous Thesis works under RGUHS and understanding rationality of the different results:**

- A Pharmaceutico – Analytical Study of Haragouri Rasa**, Nirmala V. Kannal, Shri DGM Ayurvedic Medical College, Gadag, RGUHS, Bengaluru, Karnataka. The study shows the pharmaceutical preparation of the formulation and its physical characters like colour, odour, touch, taste and a few chemical characters like pH, total ash value, acid insoluble ash, water soluble ash, loss on drying.[26],[27]
- A Comparative Pharmaceutico - Analytical Study of Haragauri Rasa Prepared by Conventional and Vertical Muffle Furnace method**, Remya. R. G., Alva's Ayurveda Medical College, Moodbidri, RGUHS, Bengaluru, Karnataka. The study shows that vertical muffle furnace is more convenient for preparation but the yield of Haragauri Rasa was more in classical method than conventional method and classical formulation shows good analytical results too.[28],[29]

After this comparison of pharmaceutical comparison, a partial agreement can be set forth that:

- ❖ Tapta Khalva can be helpful in swift preparation of Kajjali. Since, duration for preparedness of Kajjali for Rasasindooram in reference with Rasa Tarangini[30] & Rasayogasagara[31] can be seen as 2-3 Dina (12 or 24 hours for one Dina) = 24-36 hours or 48-72 hours. In the current study after 22 hours of Mardana in Tapta Khalva alone, the Kajjali Nirmana was satisfactorily done.
- ❖ To omit any doubt of Chandratva (shiny appearance), other than that of Parada (because of the nature of Loha Khalva) additional 19.5 hours of Mardana in Ashma Khalva was performed. In the end, combining both methods of Mardana added up to a total 41.5 hours which still fall in the said range.

- ❖ Temperature measurement at the neck of Koopi → physical mercury thermometers up to the range of 360°C serve as explicit option.
- ❖ Legitimate four side closed Bhatti like setup with either wood fuel or bigger gas stove results in Galastha final product.
- ❖ Antardhooma Vidhi → best to avert prolonged contact with Gandhaka fumes.

**C.) Importance of Law of definite proportion, theoretical yield, actual yield, yield percentage in any Koopipakava Nirmanam:**

- ❖ Considering all aspects, it can be postulated that as per the Law of definite proportion (by Proust in 1797) “Every chemical compound contains fixed and constant proportions by mass of its constituent elements”[32] a pre-calculation of theoretical yield percentage can be helpful in expecting how much could be the maximum amount of final product in ideal experimental condition.

e.g In every molecule of HgS the ratio of Hg and S will always be 6:1 which will be always same by mass as well.

**Importance of calculating and comparing theoretical yield:[24]**

To understand this with respect to Haragauri Rasa/Rasasindooram (HgS);

- if the prepared amount of Haragauri Rasa/Rasasindooram is exact or near to the theoretical yield, it means complete Paka of Kajjali with usual Dhooma escape in Bahirdhooma Vidhi or separation of decent amount of other Gandhaka in Antardhooma Vidhi of Koopipaka.
- if the prepared amount of prepared Haragauri Rasa/Rasasindooram is less than theoretical yield, it means Parada-Gandhaka escaped through Dhooma or Kajjali received less Agni or the set-up had lacunae.
- if the prepared amount of Haragauri Rasa/Rasasindooram is more than theoretical yield, it shows presence of excess amount of Gandhaka in the final product.

After this comparison of analytical parameters, a partial agreement can be set forth that:

- ❖ Addition of Navasadara as a Prakshepaka in Dhattura Patra Mardita Kajjali rather than as a component of Kajjali can bring exquisite Varna of final product. Thus, the role of Navasadara as Sarvaloha Ranjaka[33] can be affirmed.

- ❖ pH of the sample → Neutral to little alkaline in nature.
- ❖ Antardhooma method → more beneficial for converting the particles in minutest form of medicine.
- ❖ Irregular and porous morphology of the crystals could act as a carrier for various molecules and thus the implementation of Vyadhinusara Anupana and use of Haragauri Rasa same as Rasasindooram in Sarva Vyadhi and Yogavahi Guna can be justified.

#### Results of Anti-Microbial studies:

##### I. Disc Diffusion method and MIC:

- ❖ The sample of Haragauri Rasa was tested against pneumonia causing bacterial strains of *S. aureus* and *K. pneumonia* with standard drug Amoxicillin and was found showing significant antimicrobial activity throughout. It also showed more inhibition zone results than the amoxicillin drug at more concentration.
- ❖ The sample was also tested against pneumonia causing fungal strain of *C. albicans* with standard drug Gentamycin and was found showing significant results and found active against the fungal growth.

##### II. MIC with Dilution method:

- ❖ MIC stands for Minimum Inhibitory Concentration which suggests the minimum in-vitro levels of susceptibility or resistance of specific bacterial strains to applied medicine.
- ❖ In the present sample of Haragauri Rasa, the minimum concentrations to inhibit the bacterial growth were found to be 3.8 µg/ml and 4.6 µg/ml respectively for *K. pneumoniae* and *S. aureus* whereas the minimum required concentration to inhibit fungal growth of *C. albicans* was 7.6 µg/ml.

#### V. CONCLUSION:

The prepared sample of Haragauri Rasa shows nano-particle size of 200nm supporting Sukshmatva of Koopipakava Rasayana and their capacity to reach up to Ati Sukshma Sthana in Deha for Chikitsaphala. Haragauri Rasa shows significant results against Pneumonia causing micro-organisms as anti-bacterial and anti-fungal agent. The only bilateral principle for Siddhi (Rasa Siddhi – medicine formulation or Chikitsa Siddhi – successful treatment) is: (i) Guruseva and Shiva Smarana in all the Rasa-Nirmanana to please the

supreme power Shiva[34] and (ii) Shastra Sahita Tarka for Gyana Sadhana (accomplishment of knowledge) as per Chakrapani Teeka[35] or Karmasiddhi (successful treatment) as per Gangadhara Teeka.[36]

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